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Preparation and optical properties of new fluorescent iminocoumarins: Study of *N*-acyl-derivatives

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Abstract

Six new iminocoumarin dyes, bearing a cyano group at the 3-position and a diethylamino group at the 7-position, were synthesized. These compounds differ by the nature of the acyl substituent borne by the imino group. They were studied in CH_2Cl_2 and ethanol by UV/vis absorption and fluorescence spectroscopy. This work shows that the optical properties strongly depend on the nature of the substituent borne by the imino group, and indicates which type of substituent is favourable for a given application. It confirms that iminocoumarin derivatives could lead to a new generation of fluorescent probes, prone to easy modification of their chemical structure.

Keywords: Iminocoumarin; Fluorescence

1. Introduction

Due to its high sensitivity and specificity, fluorescence spectroscopy is of current use in fields like cellular biology, medical analysis and pollution control, where it finds new applications everyday. However, optimal efficiency is only obtained when the fluorescent molecules that are used as probes or tracers are really suitable for a given environment. Consequently, there is a constant interest in developing families of fluorescent compounds, which exhibit good optical properties and whose chemical structure can be easily modified in different ways, so that tailor-made molecular probes can be obtained in view of a precise application.

With this in mind, we recently focused on the synthesis and spectroscopic study of new iminocoumarins. Close to the family of coumarin dyes, which constitutes one of the most famous class of fluorescent compounds [1–4], iminocoumarins have not attracted much attention until now, although their colouring and fluorescent properties have been noticed [5–10]. However, iminocoumarins can be easily substituted on their imino group, thus allowing a wide panel of compounds to be prepared. For example, this type of molecule has been associated to chelating units to give a fluorescent Ca^{2+} indicator, which shows strong affinity for cell membranes owing to the lipophilic chain borne by the imino group [11,12]. Other structures, substituted by $CH{=}C(CN)_2$ on the imino group, have also been proposed as fluorescent dyes for laser or dyeing applications [13,14]. In our team, we recently showed that *N*-ethoxycarbonylation leads to an iminocoumarin derivative, whose fluorescence properties are similar to those of the corresponding coumarin in low polarity solvents, and even better in highly polar solvents [15].

The aim of the present work is to see how the nature of the substituent borne by the imino group can affect the fluorescence properties. To do so, six new compounds were synthesized (Scheme 1), differing by the substitution on the nitrogen atom. Three of them (**1**–**3**) bear substituents that do not increase the length of the unsaturated electron system. The three others bear an aromatic ring on the N–C(O) link. The aromatic moiety can be a phenyl group with electron-donor (**4**) or electron-withdrawing character (**5**), or a furyl group (**6**). All the compounds bear a diethylamino group at the 7-position. Actually, we showed that the presence of a good electron-donor group at this position enhances intramolecular electron transfer, and thus strongly favours the fluorescence properties [16], as it is also the case in

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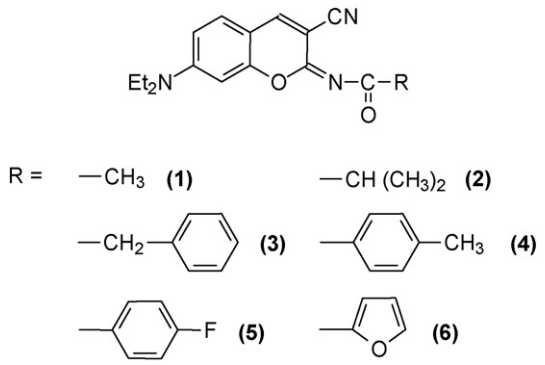


Scheme 1. Chemical structure of the iminocoumarin dyes.

the neighbouring series of coumarins. The compounds also bear a cyano group at the 3-position, the presence of which is justified by synthesis conveniences. In fact, using malononitrile for the synthesis of iminocoumarins allows these compounds to be obtained in high yields, because the presence of two nitrile groups on this reactant makes the methylenic protons very acidic. The presence of the cyano group at the 3-position reinforces the electron-withdrawing pole of the molecule, mainly played by the imino group. From a spectroscopic point of view, this leads to a red shift of the absorption and fluorescence spectra. The cyano group at this position also leads to a slight decrease of the fluorescence quantum yield and lifetime, at least in the coumarin series [16].

Dyes **1**–**6** were studied in dichloromethane and in ethanol by UV/vis absorption spectroscopy, then by steady-state and dynamic fluorescence spectroscopy.

2. Experimental

2.1. Apparatus

Melting points were determined by an Electrothermal 9100 apparatus. Infrared spectra were recorded on a Jasco FT-IR 420 spectrophotometer apparatus (in KBr pellets). ^{1}H and ^{13}C NMR spectra were obtained with a Bruker WP 200 spectrometer operating at 300 and 75 MHz, respectively, in DMSO-d_6 or $CDCl_3$ with TMS as internal standard (chemical shifts in ppm). Elemental microanalyses were performed on a EA1112 analyser from CE Instruments. High resolution mass spectra were performed on a Waters Qtof Ultima API spectrometer with electrospray probe. Phosphoric acid was used for both protonation and calibration.

All spectrophotometric measurements were conducted at 25 °C using a temperature-controlled cell holder. UV/vis absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. The estimated experimental error was 2 nm on the band maximum and 5% on the molar extinction coefficient. Steady state fluorescence work was performed on a Photon Technology International (PTI) Quanta Master 1 spectrofluorometer. All excitation and emission spectra were corrected. The fluorescence quantum yields (Φ) were determined using the classical formula: $\Phi_x = (A_s \times F_x \times n_x^2 \times \Phi_s)/(A_x \times F_s \times n_s^2)$ where A is absorbance at the excitation wavelength, F the area under the fluorescence curve, and n is the refractive index of the solvents used. Subscripts 's' and 'x' refer to the standard and to the sample of unknown quantum yield, respectively. Coumarin 6 in ethanol ($\Phi = 0.78$) was taken as the standard [17]. Fluorescence decay was measured with the stroboscopic technique utilising a Strobe Master fluorescence lifetime spectrophotometer from PTI. The excitation source was a flash lamp filled with a mixture of nitrogen and helium (30/70). Data were collected over 200 channels with a time-base of 0.1 ns per channel. Analysis of fluorescence decay was performed using the multiexponential method software from PTI.

2.2. Materials

For synthesis, acid chlorides were purchased from Aldrich. For spectroscopic measurements, analytical grade dichloromethane was from Prolabo and ethanol was from SDS.

2.3. General procedure for the synthesis of N-acyl-3-cyano-7-diethylamino-2-iminocoumarins

3-Cyano-7-diethylamino-iminocoumarin was prepared as described in ref. [18]. Then, acid chloride (10 mmol) diluted in 2 mL of chloroform was added dropwise, for 30 min, to a stirred solution of 10 mmol of iminocoumarin in 30 mL of chloroform containing 10 mmol of pyridine, while keeping the mixture at 0 °C. The basic medium was then allowed to reach room temperature and was stirred for 3–4 h. In order to remove pyridine, triple extraction (water/chloroform) was realized. After evaporation of solvent, the product was purified by precipitation in cyclohexan.

2.3.1. N-acetyl-3-cyano-7-diethylamino-2-iminocoumarin (*1*)

Yield: 68%. m.p. 138 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.22$ (t, $J = 7.2$ Hz, 6H, CH_3), 2.32 (s, 3H, CH_3), 3.43 (q, $J = 7.2$ Hz, 4H, CH_2), 6.33 (d, $J = 1.8$ Hz, 1H, H8), 6.53 (dd, $J = 8.7$ Hz, $J = 1.8$ Hz, 1H, H6), 7.20 (d, 1H, H5), 7.72 (s, 1H, H4). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 12.50$ (CH_3), 26.39 (CH_3), 45.14 (CH_2N), 94.55 (C3), 97.21 (C8), 106.53 (C6), 109.43 (C10), 115.53 (C≡N), 130.54 (C5), 147.04 (C7), 152.96 (C4), 156.33 (C9). IR (KBr) cm^{-1}: $\nu = 1663$ (C=N), 1716 (C=O), 2220 (C≡N). Anal. Calcd. for $C_{16}H_{17}N_3O_2$: C, 67.84; H, 6.00; N, 14.84%. Found: C, 66.90; H, 5.33; N, 13.99%. HRMS: Calcd. for $C_{16}H_{18}N_3O_2$: 284.1399. Found: 284.1345.

2.3.2. N-methylpropionyl-3-cyano-7-diethylamino-2-iminocoumarin (*2*)

Yield: 66%. m.p. 118 °C. ^{1}H NMR (300 MHz, DMSO): $\delta = 1.11$ (t, $J = 6.9$ Hz, 6H, CH_3), 1.11 (d, 6H, CH_3), 3.46 (q, $J = 6.9$ Hz, 4H, CH_2), 3.46 (q, 1H, CH), 6.59 (s, 1H, H8), 6.81 (d, $J = 8.7$ Hz, 1H, H6), 7.48 (d, $J = 8.7$ Hz, 1H, H5), 8.53 (s, 1H, H4). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 12.80$ (CH_3), $\delta = 12.80$ (CH_3), 44.86 (CH_2N), 44.99 (CH), 90.85 (C3), 96.98 (C8), 107.47 (C6), 110.81 (C10), 116.76 (C≡N), 131.76 (C5), 149.20 (C7), 153.36 (C4), 156.35 (C9). IR (KBr) cm^{-1}:

ν = 1627 (C=N), 1721 (C=O), 2217 (C≡N). Anal. Calcd. for $C_{18}H_{21}N_3O_2$: C, 69.45; H, 6.75; N, 13.50%. Found: C, 68.56; H, 6.12; N, 12.80%. HRMS: Calcd. for $C_{18}H_{22}N_3O_2$: 312.1710. Found: 312.1712.

2.3.3. N-(phenylacetyl)-3-cyano-7-diethylamino-2-iminocoumarin (*3*)

Yield: 70%. m.p. 180 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.24 (t, J = 8.7 Hz, 6H, CH_3), 3.34 (q, J = 8.7 Hz, 4H, CH_2), 3.90 (s, 2H, CH_3), 6.18 (d, J = 3.0 Hz, 1H, H8), 6.53 (dd, J = 10.8 Hz, J = 3.0 Hz, 1H, H6), 7.19 (d, J = 10.8 Hz, 1H, H5), 7.30 (2H, H-Ar), 7.36 (1H, H-Ar), 7.40 (2H, H-Ar), 7.72 (s, 1H, H4). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.78 (CH_3), 44.92 (CH_2N), 45.64 (CH_2), 92.26 (C3), 96.73 (C8), 107.70 (C6), 110.13 (C10), 116.32 (C≡N), 128.59 (C5), 147.49 (C7), 148.85 (C4), 153.22 (C9), 156.17 (C2), 182.82 (C=O). IR (KBr) cm^{-1}: ν = 1631 (C=N), 1721 (C=O), 2220 (C≡N). Anal. Calcd. for $C_{22}H_{21}N_3O_2$: C, 73.53; H, 5.85; N, 11.70%. Found: C, 72.56; H, 5.18; N, 11.20%. HRMS: Calcd. for $C_{22}H_{22}N_3O_2$: 360.1712. Found: 360.1747.

2.3.4. N-(4-methylbenzoyle)-3-cyano-7-diethylamino-2-iminocoumarin (*4*)

Yield: 80%. m.p. 150 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.18 (t, J = 6.9 Hz, 6H, CH_3), 2.40 (s, 3H, CH_3), 3.40 (q, J = 6.9 Hz, 4H, CH_2), 6.27 (s, 1H, H8), 6.43 (d, 1H, H6), 7.18 (d, 1H, H5), 7.24 (2H, H-Ar), 7.78 (s, 1H, H4), 8.02 (d, 2H, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.75 (CH_3), 21.70 (CH_3), 44.85 (CH_2N), 92.68 (C3), 96.65 (C8), 106.94 (C6), 110.39 (C10), 116.53 (C≡N), 129.80, 131.19 (C5), 131.82, 144.24, 149.27 (C7), 149.47 (C4), 153.37 (C9), 156.34 (C2), 175.74 (C=O). IR (KBr) cm^{-1}: ν = 1689 (C=N), 1722 (C=O), 2220 (C≡N). Anal. Calcd. for $C_{22}H_{21}N_3O_2$: C, 73.53; H, 5.85; N, 11.70%. Found: C, 72.55; H, 5.10; N, 10.82%. HRMS: Calcd. for $C_{22}H_{22}N_3O_2$: 360.1712. Found: 360.1623.

2.3.5. N-(4-fluorobenzyl)-3-cyano-7-diethylamino-2-iminocoumarin (*5*)

Yield: 88%. m.p. 161 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.22 (t, J = 8.4Hz, 6H, CH_3), 3.44 (q, J = 8.4 Hz, 4H, CH_2), 6.36 (d, J = 2.4 Hz, 1H, H8), 6.58 (dd, J = 10.8 Hz, J = 2.4 Hz 1H, H6), 7.13 (d, J = 10.2 Hz, 2H, H-Ar), 7.24 (d, J = 10.8 Hz, 1H, H5), 7.86 (s, 1H, H4), 8.22 (d, J = 10.8 Hz, 2H, H-Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.75 (CH_3), 44.85 (CH_2N), 92.79 (C3), 96.73 (C8), 107.12 (C6), 110.49 (C10), 116.40, 116.45 (C≡N), 130.69 (C5), 131.76, 132.55, 149.41 (C7), 150.34 (C4), 153.40 (C9), 156.33 (C2), 167.27 (C–F), 174.39 (C=O). IR (KBr) cm^{-1}: ν = 1640 (C=N), 1728 (C=O), 2218 (C≡N). Anal. Calcd. for $C_{21}H_{18}N_3O_2F$: C, 69.42; H, 4.96; N, 11.57%. Found: C, 68.32; H, 4.32; N, 11.32%. HRMS: Calcd. for $C_{21}H_{19}N_3O_2F$: 364.1461. Found: 364.1360.

2.3.6. N-(2-furoyl)3-cyano-7-diethylamino-2-iminocoumarin (*6*)

Yield: 76%. m.p. 185 °C. ^{1}H NMR (300 MHz, DMSO): δ = 1.05 (t, J = 7.5 Hz, 6H, CH_3), 3.40 (q, J = 7.5 Hz, 4H, CH_2), 6.28 (d, J = 1.5 Hz, 1H, H8), 6.67 (dd, J = 8.7 Hz, J = 1.5 Hz, H6), 6.68 (dd, J = 6.9 Hz, J = 1.5 Hz, 1H, CH), 7.29 (d, 1H, CH), 7.41 (d, J = 8.7 Hz, 1H, H5), 7.95 (s, 1H, H4), 8.35 (s, 1H, CH–O). ^{13}C NMR (75 MHz, DMSO): δ = 12.72 (CH_3), 45.00 (CH_2N), 44.99 (CH), 96.70 (C3), 96.88 (C8), 107.07 (C6), 110.71 (C10), 116.35 (C≡N), 131.77 (C5), 148.21 (C7), 149.49 (C4), 153.45 (C9), 156.29 (C2), 166.35 (C=O). IR (KBr) cm^{-1}: ν = 1638 (C=N), 1723 (C=O), 2222 (C≡N). Anal. Calcd. for $C_{19}H_{17}N_3O_3$: C, 68.06; H, 5.07; N, 12.54%. Found: C, 67.74; H, 4.90; N, 12.02%. HRMS: Calcd. for $C_{19}H_{18}N_3O_3$: 336.1348. Found: 336.1411.

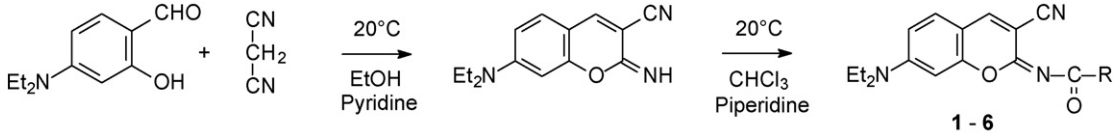
3. Results and discussion

3.1. Preparation of the compounds

The synthetic procedure was accomplished in two steps including (i) the preparation of 3-cyanoiminocoumarin followed by (ii) its *N*-acylation, as shown in Scheme 2. In the first step, 3-cyano-7-diethylamino-iminocoumarin was obtained by condensation of 4-(diethylamino)salicylaldehyde with an equimolecular amount of malononitrile in the presence of piperidine as the catalyst, as previously described [18]. This one-pot reaction led to the desired product with a yield of 60%. Then, in the second step, the substitution of the imino group was achieved by reacting 3-cyano-7-diethylamino-iminocoumarin with a suitable acid chloride in the presence of pyridine. Interestingly, the presence of a *N*-diethylamino group in the 7-position increased the reactivity of the imino group, due to the strong electron donating effect that is transmitted by charge transfer. Compounds **1**–**6** were obtained in high yields, ranging between 66 and 88%. It can be noticed that the development of this reaction is influenced by the structure of the acid chloride used. Actually, aromatic acid chlorides appeared to be more reactive (yield: 76–88%) than aliphatic ones (yield: 66–70%). The structure of compounds **1**–**6** was assigned by IR, ^{1}H and ^{13}C NMR spectroscopy, and high resolution mass spectrometry.

3.2. UV/vis absorption spectra

For UV/vis absorption measurements, the dye concentration was between 2×10^{-5} and 3.5×10^{-5} M. The absorption spectrum of all the dyes in dichloromethane and ethanol showed a



Scheme 2. Reactions and conditions. The R groups are detailed in Scheme 1.

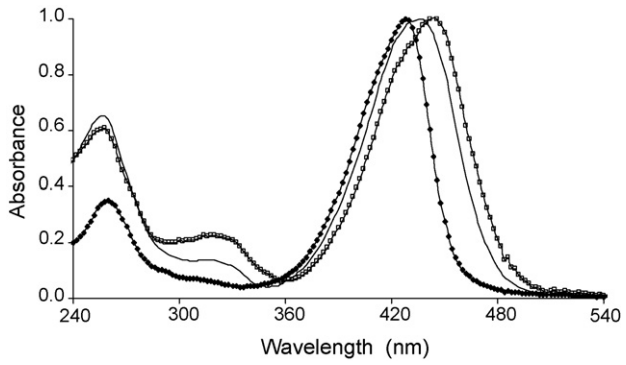


Fig. 1. Absorption spectrum of compounds **2** (2.2×10^{-5} M, diamonds), **4** (3.2×10^{-5} M, plain line) and **5** (2.6×10^{-5} M, squares) in dichloromethane.

weak band around 260 nm (π–π* transition) and an intense and rather symmetrical band at long wavelengths, as illustrated in Fig. 1. This band can be attributed to the charge transfer that takes place between the electron-donor group (diethylamino group) and the electron-withdrawing pole (cyano and iminoacyl groups) of the molecule. The absorption spectra of compounds **1**, **2**, **3** and **6** were almost superimposable, peaking at around 430 nm in dichloromethane. In contrast, compounds **4** and **5** that bear a phenyl ring directly on the N–C(O) group absorbed at longer wavelengths than the other compounds (Table 1). Therefore, it seems that the presence of an arylcarbonyl group on the imino function increases the conjugated electron system. The red shift was particularly strong for fluorinated compound **5**, and was attributed to the presence of a strong electron-withdrawing atom that enhances intramolecular charge transfer. The values of the molar extinction coefficients (ε) were measured for compounds **2**, **4** and **5**. They varied between 31,000 and 45,000 $M^{-1}cm^{-1}$. This difference seems quite large, and it was surprising to see that compounds **4** and **5** with extended conjugated electron system actually had a smaller value of ε than the isopropyl derivative **2**. However, it must be noticed that the absorption band was much wider for **4** and **5** than for **2**, as measured by the full width at half-maximum value (Table 1), and illustrated in Fig. 1. It can be noted that the absorption spectrum of all the compounds was slightly shifted to the blue when passing from dichloromethane to ethanol. Considering the solvatochromic behaviour of closely related molecules [15], this effect was attributed to a specific solvent effect occurring with dichloromethane.

3.3. Excitation spectra

For fluorescence spectroscopy, the dye concentration was between 1.1×10^{-6} and 1.6×10^{-6} M, so absorbance at the excitation wavelength was kept around 0.05. For all compounds, the excitation spectrum was similar to the absorption spectrum, and did not vary with the emission wavelength. This indicates that the species visible on the absorption spectrum is also responsible for fluorescence emission.

3.4. Steady-state emission spectra

For all compounds, the shape and position of the emission spectra were independent of the excitation wavelength, which confirms that only one species emits in each solution. The emission spectra showed one asymmetric emission band without vibrational structure (see Fig. 2 for an example). In dichloromethane, the emission maximum was situated around 460 nm for compounds **1**, **2** and **3** and **6**. In contrast, compounds **4** and **5** emitted above 500 nm (Table 1). In ethanol, the emission maximum was markedly shifted to the red for all compounds, and the Stokes shift ($\nu_{abs} - \nu_{em}$) increased drastically, especially for compound **4**. This behaviour called positive solvatochromism indicates that the excited state is more polar than the ground state [19]. It was thoroughly studied in a previous work for 3-cyano-7-diethylamino-iminocoumarin and its

Table 1
Spectroscopic and photophysical characteristics of the iminocoumarin derivatives in dichloromethane (top) and in ethanol (bottom)

Compound	λ_{abs} (nm)	ε (M^{-1} cm^{-1})	FWHM (cm^{-1})	λ_{em} (nm)	$\nu_{abs} - \nu_{em}$ (cm^{-1})	Φ	τ (ns)	k_r (10^8 s^{-1})	k_{nr} (10^8 s^{-1})
In dichloromethane									
1	430	–	2717	460	1517	0.80 ± 0.02	4.0 ± 0.2	2.00 ± 0.16	0.50 ± 0.08
2	428	45400	2717	460	1625	0.83 ± 0.03	3.1 ± 0.1	2.68 ± 0.20	0.55 ± 0.12
3	430	–	2591	460	1517	0.87 ± 0.03	3.0 ± 0.2	2.90 ± 0.31	0.43 ± 0.14
4	436	31000	3371	502	3015	0.76 ± 0.02	3.9 ± 0.1	1.95 ± 0.10	0.62 ± 0.07
5	444	39000	3202	507	2799	0.83 ± 0.02	3.8 ± 0.2	2.18 ± 0.18	0.45 ± 0.08
6	430	–	2591	460	1517	0.90 ± 0.03	3.0 ± 0.1	3.00 ± 0.20	0.33 ± 0.11
In ethanol									
1	422			462	2051	0.15 ± 0.02	1.8 ± 0.2	0.83 ± 0.23	4.72 ± 0.71
2	422			470	2420	$4.7 (\pm 0.2) \times 10^{-2}$	<0.4[a]	–	–
3	424			472	2398	$3.3 (\pm 0.2) \times 10^{-2}$	<0.4[a]	–	–
4	430			530	4388	0.40 ± 0.03	3.2 ± 0.1	1.25 ± 0.14	1.88 ± 0.16
5	442			533	3863	0.30 ± 0.03	3.0 ± 0.1	1.00 ± 0.13	2.33 ± 0.19
6	424			470	2308	$3.3 (\pm 0.2) \times 10^{-2}$	<0.4[a]	–	–

λ_{abs}: maximum absorption wavelength of the CT band; ε: corresponding molar extinction coefficient; FWHM: full width at half maximum; λ_{ex}: maximum excitation wavelength; λ_{em}: maximum emission wavelength and shoulders; $\nu_{abs} - \nu_{em}$: Stokes shift; Φ_f: fluorescence quantum yield with excitation at the maximum absorption wavelength; τ: fluorescence lifetime; k_r and k_{nr}: radiative and non-radiative deactivation constants, respectively.

[a] The τ value must be considered with circumspection, since our apparatus lacks precision below 0.7 ns.

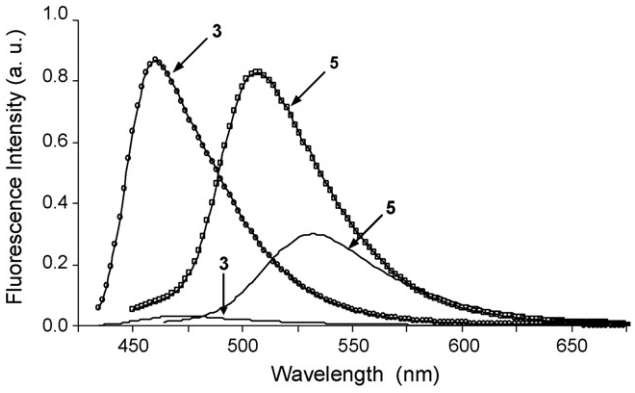


Fig. 2. Emission spectrum of compounds **3** (1.1×10^{-6} M) and **5** (1.3×10^{-6} M) in dichloromethane (circles and squares, respectively) and ethanol (plain line). Excitation at the absorption maximum. The intensity at the emission maximum is proportional to fluorescence quantum yield.

N-ethoxycarbonyl derivative, placed in a large number of solvents [15]. The *N*-acyl derivatives studied in the present work behave on a similar way.

3.5. Fluorescence quantum yields

The fluorescence quantum yield was determined by excitation at the absorption maximum for each dye. In dichloromethane, all the dyes exhibited a high quantum yield, ranging from 0.76 to 0.90 (Table 1). It can be noted that this quantum yield was similar to that of 7-diethylamino-3-cyano-iminocoumarin and to that of 7-diethylamino-3-cyano-coumarin in the same solvent [15]. In ethanol, the quantum yield of all the dyes was markedly reduced. That of dyes **2**, **3** and **6** became quite low (between 3 and 4×10^{-2}). Curiously, dye **1** was more fluorescent in ethanol than its analogues that bear an isopropyl or benzyl group. Finally, the fluorescence quantum yield of **4** and **5** remained at an interesting value (0.40 and 0.30, respectively).

3.6. Fluorescence lifetimes

The fluorescence lifetimes were measured by excitation at 337 nm, collecting the signal at the maximum emission wavelength for each compound. The decays were found to be mono-exponential in every case and it was checked that using two exponentials did not improve the fit. In dichloromethane, the fluorescence lifetime of all the dyes was between 3 and 4 ns. In ethanol, the fluorescence lifetime was reduced with respect to that in dichloromethane. It remained around 3 ns for dyes **4** and **5**, but was decreased by half for compound **1**. For dyes **2**, **3** and **6**, the fluorescence lifetime was so markedly reduced that it was too short to be measured accurately by our apparatus.

3.7. Deactivation constants

The values of the fluorescence quantum yield and lifetime give access to the calculation of the radiative (k_r) and non-radiative (k_{nr}) deactivation constants, classically defined as $k_r = \Phi/\tau$ and $k_{nr} = (1 - \Phi)/\tau$. Let us recall that, very schematically, a high value for k_r indicates that the energy levels of the molecule favour high fluorescence efficiency, whereas a high value for k_{nr} indicates that non-radiative deactivations, such as rotations, vibrations, or hydrogen bonding, take place in the molecule and offer deactivation channels that compete with fluorescence.

The analysis of the deactivation constants obtained for the dyes in dichloromethane does not bring much information. It only confirms that all the compounds behave similarly in this solvent. In ethanol, the deactivation constants could not be calculated for compounds **2**, **3** and **6**. However, for the other three compounds, it can be noted that passing from dichloromethane to ethanol leads to a marked increase of the non-radiative deactivation constants, and a moderate decrease of the radiative deactivation constants. This could indicate that the fluorescence quenching action of the solvent is quite complex. The energy levels can be varied in a polar solvent so that the gap between them is reduced and non-radiative deactivation is favoured. Hydrogen bonds can also form due to solvent proticity.

4. Conclusions

This spectroscopic study was performed in only two solvents, chosen to give an overall idea of the dyes' behaviour in different media. For more information on the solvatochromic behaviour of iminocoumarin dyes, the reader is invited to refer to one of our previous work, where it was analysed in water and in 18 different organic solvents [15].

This work shows that the nature of the substituent introduced on the imino function is of major importance for the spectroscopic behaviour. The analysis of the absorption and emission data revealed that the dyes investigated could be roughly divided into two groups. One group is formed by dyes **4** and **5** that bear a phenyl group directly linked to the NC(O) group. These compounds are characterized by red-shifted absorption and emission spectra, marked solvatochromic effect, and good fluorescence efficiency, even in polar and protic medium. It can be noted that the fluorescence efficiency of compounds **4** and **5** in ethanol is comparable to that of the analogue non-substituted on the imino function ($\Phi = 0.33$), and 10 times higher than that of the corresponding coumarin ($\Phi = 0.034$) [15]. Compounds such as **4** and **5** can then be used as fluorescent labels that should provide good fluorescence signal in very different environments.

Compounds **1**, **2**, **3** and **6** constitute the second group of dyes, although dye **1**, by its behaviour, is somewhat intermediate between the two groups. Three of the compounds (**1**–**3**) bear a CH_2 group directly on the NC(O) group, so that electron delocalisation is prevented. Unexpectedly, compound **6** that bears an unsaturated furyl group displays a similar behaviour. An explanation could be that the furyl group is not planar with respect to the rest of the molecule and electrons cannot delocalise. The particular interest of dyes **2**, **3** and **6** is that their fluorescence was almost totally quenched in a polar and protic medium. This indicates that this type of compound can advantageously be used as a fluorescent probe for lipid phases such as membranes in cell biology. If necessary, the lengthening of the fatty chain should be easily achieved.

In conclusion, 7-diethylamino-3-cyano-iminocoumarins could be the building block of a new generation of fluorescent probes, which combine the excellent optical properties of coumarins with real simplicity of synthesis.

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References

[1] R.M. Christie, Rev. Prog. Color. 23 (1993) 1.
[2] K.H. Drexhage, in: F.P. Schäfer (Ed.), Dye Lasers. Topics in Applied Physics, vol. 1, Springer, Berlin, 1973.
[3] B.M. Krasovitskii, B.M. Bolotin, Organic Luminescent Materials, VCH, Weinheim, 1988.
[4] H. Schwander, P. Hendrix, in: Y.S. Yamamoto (Ed.), Ullmann's Encyclopedia of Industrial Chemistry, vol. A11, fifth ed., VCH, Weinheim, 1988, pp. 280–281.
[5] A.A. Karasev, L.L. Lukatskaya, M.I. Rubtsov, E.K. Zhikol, S.N. Yarmolenko, O.A. Ponomarev, Russ. J. Gen. Chem. 65 (1995) 1416.
[6] A.A. Karasyov, Z.A. Sizova, A.O. Doroshenko, V.V. Maslov, L.L. Lukatskaya, M.I. Rubtsov, Funct. Mater. 9 (2002) 242.
[7] V.V. Maslov, M.I. Dzyubenko, V.M. Nikitchenko, Kvantovaya Elektronika 16 (1989) 709.
[8] T. Ooyama, Y. Shimokawa, I. Aida, H. Koshida, H. Takuma, R. Takigushi, H. Eguchi, M. Kafuku, Jpn. Kokai Tokkyo Koho, JP 05169853 (1993). Chemical Abstracts 119 (1993) 214041.
[9] Y.V. Bilokin, M.V. Vasylyev, O.V. Branytska, S.M. Kovalenko, V.P. Chernykh, Tetrahedron 55 (1999) 13757.
[10] C. O'Callaghan, T.B.H. McMurry, J.E. O'Brien, S.M. Draper, D.J. Wilcoc, J. Chem. Soc. Perkin Trans. 1 10 (1996) 1067.
[11] F. Liepouri, T.G. Deligeorgiev, Z. Veneti, C. Savakis, H.E. Katerinopoulos, Cell Calcium 31 (2002) 221.
[12] F. Liepouri, E. Foukaraki, T.G. Deligeorgiev, H.E. Katerinopoulos, Cell Calcium 30 (2001) 331.
[13] P. Czerney, H. Hartmann, Patent DD 221191 (1985). Chemical Abstracts 104 (1986) 111374.
[14] N.B. Desai, Ger. Offen, DE 2717599 (1977). Chemical Abstracts 88 (1978) 75311.
[15] H. Turki, S. Abid, R. El Gharbi, S. Fery-Forgues, C.R. Chimie, in press.
[16] H. Turki, S. Abid, S. Fery-Forgues, R. El Gharbi, Dyes Pigm., in press.
[17] G.A. Reynolds, K.H. Drexhage, Opt. Commun. 13 (1975) 222.
[18] H. Turki, S. Abid, Y. Le Bigot, S. Fery-Forgues, R. El Gharbi, Synth. Commun. 34 (2004) 3553.
[19] P. Suppan, N. Ghoneim, Solvatochromism, The Royal Society of Chemistry, Cambridge, 1997.